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Recent Insights Emerging from Malignant Mesothelioma Genome Sequencing

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In this issue, Lo Iacono et al. present a retrospective study performed on 123 formalin-fixed, paraffin-embedded (FFPE) biopsies from 123 malignant mesothelioma (MM) patients, using next generation sequencing (NGS) with a commercially available library of genes that are frequently mutated in cancers. Biopsies were sequenced with the Ion Torrent platform, and positive results were validated by Sanger sequencing.[1] Two limitations of this approach, acknowledged by the authors, are as follows: (1) as *BAP1* and *NF2* were not represented in the commercial library, their mutations status was analyzed using a different custom-made library and (2) the particular targeted NGS methodology chosen in this article specifically detects small gene mutations, whereas this methodology cannot detect large genetic losses, gene amplifications and translocations, or epigenetic changes.

In a parallel study recently published in Cancer Research, Guo et al.[2] performed whole exome sequencing on DNA and matched blood samples of 22 MM frozen biopsies from 22 patients collected in the operating room, integrated with the analysis of somatic copy number alterations. Exome capture libraries were sequenced on the Illumina HiSeq platform.[2] In both studies, the biopsies were from patients previously treated with chemotherapy (100% of patients Lo Iacono et al. and 41% Guo et al.). Therefore, the well-documented mutagenic effects of chemotherapy on the cancer genome[3] may have contributed to some of the mutations detected.

These studies[1,2] present the first landscape view of the somatic genomic alterations in MM. Because the researchers[1,2] used different experimental approaches, an exact comparison of the results is not possible. However, concordant data developed by independent research teams based in Europe and in the US, using different experimental strategies, provide reassurance on the reliability of these results. In fact, these two studies, which used robust statistical algorithms for the mutation analysis, show minor discrepancies and several corroborating findings.

In both studies, the number of nonsynonymous gene mutations was smaller than in other cancers. Lo Iacono et al. found that 20 of 52 "cancer" genes studied harbored variations in 25 of 123 (20%) of FFPE biopsies (including intronic, synonymous, nonsynonymous, and regulative mutations). These mutations were clustered in the two main "p53/DNA repair" (*TP53*, *SMACB1*, and *BAP1*) and "PI3K-AKT" (*PDGFRA*, *KIT*, *KDR*, *HRAS*, *PIK3CA*, *STK11*, and *NF2*) pathways.

Guo et al. instead sequenced "only" the exome, but looked for all possible genes and found 490 mutated genes, of which 447 (97%) were mutated only in one biopsy, and found an average of 23 mutations per biopsy (range 2–51).

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Several factors may explain the different mutation frequencies detected: the different sources of DNA (FFPE[1] vs. frozen biopsies[2]), different platforms, study design and algorithms used, and the fact that Lo Iacono et al.[1] sequenced also the intronic regions (except for *BAP1* and *NF2*). The genes most often mutated in both studies were *BAP1*, *NF2*, and *CDKN2A*.

Both studies detected frequent somatic nonsynonymous *BAP1* mutations in 41%[2] and 58%[1] of MMs, respectively, confirming previous findings that detected somatic *BAP1* mutations in 22–61% of MMs.[4–6] These mutations result in stop codons that produce truncated BAP1 proteins lacking the nuclear localization sequence or are mutations within the catalytic subunit that impair BAP1 autodeubiquitination, which is required for nuclear localization.[7] Therefore, these mutations are predicted to result in BAP1 proteins that cannot migrate to the nucleus and which may have aberrant deubiquitinase activity in the cytoplasm.[8] Accordingly, Lo Iacono et al. found that 52% of 116 MM biopsies stained for nuclear BAP1—an indication of normal BAP1 activity—whereas 48% did not, an indication of mutated BAP1.[1] Nuclear BAP1 staining correlated with presence/absence of DNA mutations ($p = 0.001$).

Both studies[1,2] report frequent mutations of *NF2*, encoding Merlin, a component of the Hippo signaling pathway,[9] with about 50% frequency, a value comparable with previous reports.[10,11] Surprisingly, 92% of the specimens analyzed by Lo Iacono et al. showed NF2 expression by immunohistochemistry.[1] The authors propose that the genetic variations detected might deregulate NF2 without affecting protein expression and stability. Alternatively, the *NF2* genetic mutations detected are often of minor biological significance.

Lo Iacono et al. emphasize the possible role of mutations found in the *PIK3CA* gene encoding the catalytic subunit of PI3K in favoring tumor progression. However, the authors acknowledge the need to study larger cohorts before conclusions can be drawn.

Guo et al. performed an integrative pathway analysis of somatic mutations and focal somatic copy number alterations: most recurrent alterations were in the MAPK and Wnt signaling pathways and in the cell cycle, with recurrent alterations of *CUL1*, *CDKN2A*, and *TP53*, the last two matching the results obtained by Lo Iacono et al.

In summary, despite the different study designs and methodologies, these two NGS analyses of the MM genome reveal that inactivating mutations occur randomly and are rarely shared among MM biopsies, with the exception of *BAP1*[1,2] and to a lesser extent *NF2*, *CDKN2A*,[1,2] and possibly *CUL1*.[2]

These results are in agreement with a large body of research that led to the conclusion that driver mutations (i.e., gene mutations present in all tumor cells in most cancers of the same type) are rare; a finding that significantly complicates the attempt to develop target therapies. In fact, the main goal of NGS tumor studies is to identify somatic driver mutations that would become potential therapeutic targets and/or clinical biomarkers. Instead, the emerging picture indicates that each single tumor has its own specific sets of genetic alterations. In addition to intertumor genetic heterogeneity, there is also significant intratumor genetic heterogeneity, as branched evolutionary growth generates genetic diversity in several tumor subclones. Thus, a single biopsy taken at a given time point is unlikely to be representative of the full spectrum of tumor genetic alterations. For example, over 60% of mutations detected by NGS in clear cell carcinomas of the kidney were not present in all tumor areas sampled.[12] In the same study, mTOR mutations were found in seven of eight primary sites, but in none of three metastases.[12] The studies of Lo Iacono et al. and of Guo et al. did not explore the issue of intratumor genetic heterogeneity. Based on studies in other tumor types,[12] and the recent findings that MMs are heterogeneous from start because they originate as polyclonal malignancies,[13] it is easy to predict that MMs will also show marked intratumor heterogeneity, further complicating attempts to develop molecular therapies that may benefit a large number of patients.

However, and despite these considerations, the studies of Lo Iacono et al. and Guo et al. have succeeded in identifying recurrent genetic alterations in MMs that may be "actionable," *BAP1* being the most common. BAP1 is a nuclear ubiquitin carboxy-terminal hydrolase, associated with multiprotein complexes regulating key cellular pathways, including the cell cycle, cellular differentiation, cell death, gluconeogenesis, and DNA repair.[8] When mutated in the germline, carriers develop MMs, uveal and cutaneous melanomas, renal and cholangiocarcinomas, other malignancies, and often several cancers in combination.[5,8] Moreover, several studies point at *BAP1* as harboring the putative driver mutations for sporadic (nongenetically related) MMs. We[5] and Bott et al.[4] initially reported—in independent and parallel studies using Sanger sequencing from MM biopsies—that 22% and 23% of MM biopsies contained somatic *BAP1* mutations. Yoshikawa et al.[6] reported that 61% of cell cultures derived from MM biopsies contained *BAP1* mutations. Arzt et al.[14] found that 60% of MM did not stain for nuclear BAP1, suggestive of inactivating mutations. To address the discrepancy in the frequency of *BAP1* mutations detected in different studies, we used an integrated genomic approach to study frozen MM biopsies, which included Sanger sequencing, Multiplex Ligation-Dependent Probe Amplification, cDNA sequencing, copy number analyses, methylation studies of the *BAP1* promoter, and immunohistochemistry. We found that 14 of 22 (63.6%) MM biopsies contained *BAP1* mutations/inactivation. None of these methodologies alone was able to capture all inactivating BAP1 mutations. Thus, studies using methodology based exclusively on one type of molecular approach, such as Sanger sequencing[4,5] or NGS,[1,2] will underestimate the percent of MMs carrying *BAP1* mutations. However, BAP1 nuclear staining was detected only in the eight specimens demonstrated to contain wild-type *BAP1* by the integrated genomic approach described above. Therefore, immunohistochemistry appeared to be capable of capturing the whole array of possible mechanisms of BAP1 inactivation.[15]

Several findings underscore the apparent "driver" role of *BAP1* in MMs and point at BAP1 as a potentially useful target: (1) multiple studies, including these two recent NGS[1,2] studies, found that *BAP1* is frequently mutated in MM; (2) the persistence of *BAP1* mutations in early lesions and in

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MM biopsies established in cell culture, suggesting that BAP1 mutations are an early event and that MM cells do not select against BAP1 mutations[8]; (3) the finding that, with rare exceptions, BAP1 nuclear staining is either detected in 100% of MM cells or it is not detected at all.[15] Moreover, frequent somatic mutations in *BAP1* are present in several malignancies,[8] making therapies to restore BAP1 activity in tumors relevant to many cancer patients.

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